

In re Application of:  
Rheins and Morhenn  
Application No.: 09/375,609  
Filed: August, 17, 1999  
Page 2

PATENT  
Attorney Docket No.: DERM1100-1

**Amendments to the Claims:**

Please cancel claims 76, 77, 85-87, 149-156, 160, and 163, without prejudice or disclaimer.

Please amend claims 64, 78, and 80-83, as indicated in the Listing of Claims.

**Listing of Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

Claims 1-10 Canceled

11. (Withdrawn) A method of distinguishing an irritant contact dermatitis (ICD) from an allergic contact dermatitis (ACD) in a subject, comprising, quantifying a polynucleotide level encoding a cytokine, wherein the polynucleotide level determines whether the dermatitis is ICD or ACD.
12. (Withdrawn) The method of claim 11, wherein the polynucleotide is RNA or DNA.
13. (Withdrawn) The method of claim 12, wherein the RNA is mRNA.
14. (Withdrawn) The method of claim 11, wherein the subject is a human.
15. (Withdrawn) The method of claim 11, wherein the polynucleotide is from the cells below the stratum corneum of the skin, the method further comprising:
  - (a) removing the stratum corneum; and
  - (b) collecting polynucleotide from the surface exposed after removal of the stratum corneum.

In re Application of:  
Rheins and Morhenn  
Application No.: 09/375,609  
Filed: August, 17, 1999  
Page 3

PATENT  
Attorney Docket No.: DERM1100-1

16. (Withdrawn) The method of claim 15, wherein removal of the stratum corneum uses procedures selected from the group consisting of:

- (a) abrading the stratum corneum; and
- (b) contacting the stratum corneum with an adhesive surface.

17. (Withdrawn) The method of claim 15, wherein the polynucleotide is collected from the surface exposed after removal of the stratum corneum using a procedure selected from the group consisting of:

- (a) scraping the surface exposed with a rigid surface; and
- (b) contacting the surface exposed with an adhesive surface.

18. (Withdrawn) The method of claim 17, wherein the adhesive surface comprises adhesive tape.

19. (Withdrawn) The method of claim 13, wherein the mRNA is specific for a cytokine.

20. (Withdrawn) The method of claim 19, wherein the cytokine is IL-4 and IL-8.

21. (Withdrawn) The method of claim 20, wherein the absence of IL-4 in the presence of a reaction is characteristic of ICD.

22. (Withdrawn) The method of claim 20, wherein the level of increase in IL-8 is indicative of the severity of ICD.

23. (Withdrawn) The method of claim 19, wherein the cytokine is IL-4.

In re Application of:  
Rheins and Morhenn  
Application No.: 09/375,609  
Filed: August, 17, 1999  
Page 4

PATENT  
Attorney Docket No.: DERM1100-1

24. (Withdrawn) The method of claim 23, wherein an increase in IL-4 is characteristic of ACD.

25. (Withdrawn) The method of claim 24, wherein the level of increase in IL-4 is indicative of the severity of ACD.

26. (Withdrawn) The method of claim 11, further comprising exposing the skin to a factor prior to isolating the polynucleotide.

27. (Withdrawn) The method of claim 26, wherein the factor is an irritant, antigen or allergen.

28. (Withdrawn) A method of diagnosing ICD in a subject, comprising quantifying a polynucleotide encoding a cytokine selected from the group consisting of IL-4 and IL-8 in cells isolated from the subject, wherein the amount of IL-4 or IL-8 is indicative of ICD.

29. (Withdrawn) The method of claim 28, wherein the polynucleotide is detected by PCR.

30. (Withdrawn) The method of claim 28, wherein the polynucleotide is detected by hybridization with a polynucleotide probe.

31. (Withdrawn) The method of claim 28, wherein the polynucleotide is detected by RNase protection assay.

32. (Withdrawn) The method of claim 28, wherein the cells are skin cells.

In re Application of:  
Rheins and Morhenn  
Application No.: 09/375,609  
Filed: August, 17, 1999  
Page 5

PATENT  
Attorney Docket No.: DERM1100-1

33. (Withdrawn) The method of claim 28, wherein the subject is a mammal.
34. (Withdrawn) The method of claim 33, wherein the mammal is a human.
35. (Withdrawn) A method of diagnosing ACD in a subject, comprising quantifying a polynucleotide encoding IL-4 in cells of the subject, wherein an elevated amount of IL-4 is indicative of ACD.
36. (Withdrawn) The method of claim 35, wherein the IL-4 is detected by PCR.
37. (Withdrawn) The method of claim 35, wherein the IL-4 is detected by hybridization with a polynucleotide probe.
38. (Withdrawn) The method of claim 35, wherein the IL-4 is detected by RNase protection assay.
39. (Withdrawn) The method of claim 35, wherein the cells are skin cells.
40. (Withdrawn) The method of claim 35, wherein the subject is a mammal.
41. (Withdrawn) The method of claim 40, wherein the mammal is a human.
42. (Withdrawn) A method of identifying a compound which causes a dermatitis, comprising contacting a section of skin with the compound under conditions which would induce a dermatitis and detecting a polynucleotide encoding a cytokine wherein the presence of the polynucleotide is indicative of a dermatitis.

In re Application of:  
Rheins and Morhenn  
Application No.: 09/375,609  
Filed: August, 17, 1999  
Page 6

PATENT  
Attorney Docket No.: DERM1100-1

43. (Withdrawn) The method of claim 42, wherein the compound is an allergen.
44. (Withdrawn) The method of claim 42, wherein the compound is an irritant.
45. (Withdrawn) The method of claim 42, wherein the dermatitis is allergic contact dermatitis (ACD).
46. (Withdrawn) The method of claim 42, wherein the dermatitis is irritant contact dermatitis (ICD).
47. (Withdrawn) The method of claim 42, wherein the skin is contacted *in vivo*.
48. (Withdrawn) The method of claim 42, wherein the skin is contacted *in vitro*.
49. (Withdrawn) The method of claim 42, further comprising isolating polynucleotides from the skin.
50. (Withdrawn) The method of claim 49, wherein the polynucleotides are DNA or RNA.
51. (Withdrawn) The method of claim 50, further comprising quantifying a polynucleotide encoding IL-4, wherein an elevated amount of IL-4 is indicative of ACD.
52. (Withdrawn) The method of claim 50, further comprising quantifying a polynucleotide encoding a cytokine selected from the group consisting of IL-4 and IL-8 in cells isolated from the subject, wherein the amount of IL-4 or IL-8 is indicative of ICD.

53. (Withdrawn) The method of claim 52, wherein an increase in IL-8 in the absence of IL-4 is indicative of ICD.

54. (Withdrawn) A method of diagnosing ACD in a subject, comprising quantifying a polynucleotide encoding IL-13 in cells of the subject, wherein an elevated amount of IL- 13 is indicative of ACD.

55. (Withdrawn) The method of claim 54, wherein the IL-13 is detected by PCR.

56. (Withdrawn) The method of claim 54, wherein the IL-13 is detected by hybridization with a polynucleotide probe.

57. (Withdrawn) The method of claim 54, wherein the IL-13 is detected by RNase protection assay.

58. (Withdrawn) The method of claim 54, wherein the cells are skin cells.

59. (Withdrawn) The method of claim 54, wherein the subject is a mammal.

60. (Withdrawn) The method of claim 59, wherein the mammal is a human.

61. (Withdrawn) A kit for obtaining polynucleotides from the skin, the kit comprising:

a cell collection device selected from the group consisting of a rigid surface and an adhesive tape; and

a cell lysis buffer suitable of preserving polynucleotides or a computer chip suitable for preserving polynucleotides.

62. (Withdrawn) The kit of claim 61, which further comprises an mRNA detection reagent.

63. (Withdrawn) A kit for distinguishing an irritant reaction from an allergic reaction, the kit comprising a cell collection device, a cell lysis buffer, an mRNA detection reagent.

1 ~~64~~ (Currently Amended) A method for ~~isolating or detecting~~ quantitating relative expression of a nucleic ribonucleic acid (RNA) sample from a skin sample, the method comprising:

(a) applying an adhesive tape to the skin ~~and removing the adhesive tape from the skin between one and twelve times, under conditions allowing for isolation of thereby isolating a~~ skin sample adhering to the adhesive tape, ~~wherein the skin sample comprises a nucleic acid; and~~

(b) <sup>detecting</sup> ~~isolating or detecting~~ the nucleic acid RNA from the skin sample; and

(c) comparing the level of the RNA in the skin sample to a control sample, thereby quantitating relative expression of the RNA.

2 ~~65~~ (Previously Presented) The method of claim ~~64~~, wherein the skin sample comprises stratum corneum cells and cells associated with the stratum corneum which are removed by application and removal of the adhesive tape.

70. (Canceled)

71. (Previously Presented) The method of claim 64, wherein the skin sample is isolated by applying the adhesive tape to the skin between one and two times to obtain the skin sample.

72. (Previously Presented) The method of claim 64, wherein the sample is isolated by one application of an adhesive tape to an outer layer of the skin.

Claims 73-77 (Canceled)

3 ~~78.~~ (Currently Amended) The method of claim ~~77~~ <sup>1</sup> ~~64~~, wherein the <sup>detecting</sup> ~~isolating or~~ detecting comprises <sup>detecting</sup> ~~isolating or detecting an RNA that comprises an mRNA.~~

79. (Canceled)

5 ~~80.~~ (Currently Amended) The method of claim ~~149~~ <sup>1</sup> ~~64~~, wherein the <sup>detecting</sup> ~~isolating or~~ detecting comprises <sup>detecting</sup> ~~isolating or detecting a nucleic acid an RNA~~ that encodes a cytokine.

6 ~~81.~~ (Currently Amended) The method of claim ~~149~~ <sup>1</sup> ~~64~~, wherein the <sup>detecting</sup> ~~isolating or~~ detecting comprises <sup>detecting</sup> ~~isolating or detecting a nucleic acid an RNA~~ that encodes an interleukin.

7 ~~82.~~ (Currently Amended) The method of claim ~~149~~ <sup>1</sup> ~~64~~, wherein the <sup>detecting</sup> ~~isolating or~~ detecting comprises <sup>detecting</sup> ~~isolating or detecting a nucleic acid an RNA~~ that encodes interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-3 (IL-3), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-12 (IL-12), interleukin-13 (IL-13), granulocyte macrophage colony stimulating factor (GM-CSF), or an interferon, or any combination thereof.



In re Application of:  
Rheins and Morhenn  
Application No.: 09/375,609  
Filed: August, 17, 1999  
Page 10

PATENT  
Attorney Docket No.: DERM1100-1

4 ~~83~~ (Currently Amended) The method of claim <sup>3</sup> ~~78~~, wherein the <sup>detecting</sup> ~~isolating or detecting~~ comprises <sup>detecting</sup> ~~isolating or detecting a nucleic acid~~ an RNA that encodes an inflammatory mediator.

Claims 84-156 (Canceled)

8 ~~157~~ (Previously Presented) The method of claim <sup>1</sup> ~~64~~, wherein the adhesive tape is applied to skin from a subject afflicted with a disease, disorder, or inflammatory reaction.

9 ~~158~~ (Previously Presented) The method of claim <sup>8</sup> ~~157~~, wherein the adhesive tape is applied to skin from a subject afflicted with dermatitis.

10 ~~159~~ (Previously Presented) The method of claim <sup>1</sup> ~~64~~, wherein the method further comprises contacting the skin with an external agent that causes dermatitis before applying the adhesive tape to the skin.

160. (Canceled)

161. (Previously Presented) The method of claim 64, wherein the adhesive tape is applied and removed from the skin to isolate a skin sample that comprises cells associated with the stratum corneum.

162. (Canceled)

163. (Canceled)

164. (Currently Amended) The method of claim ~~149~~ 64, wherein the adhesive tape is applied and removed between one and twelve times to isolate the skin sample.

165. (New) The method of claim 64, wherein between one and twelve separate adhesive tape strips are applied to the skin to isolate the skin sample.

Claims 165 and 166 appear to be identical

166. (New) The method of claim 64, wherein between one and twelve separate adhesive tape strips are applied to the skin to isolate the skin sample.

167. (New) A method for quantitating relative expression of a ribonucleic acid (RNA) from a skin sample, comprising:

- (a) applying an adhesive tape to the skin, under conditions allowing for isolation of a skin sample adhering to the adhesive tape;
- (b) isolating RNA from other nucleic acids in the skin sample; and
- (c) comparing the level of the RNA in the skin sample to a control sample, thereby quantitating relative expression of the RNA.

168. (New) The method of claim 167, wherein the skin sample is isolated by applying the adhesive tape to the skin between one and twelve times to obtain the skin sample.

169. (New) The method of claim 167, wherein the skin sample is isolated by applying the adhesive tape to the skin between one and two times to obtain the skin sample.

170. (New) The method of claim 167, wherein the skin sample is isolated by applying the adhesive tape to the skin one time to obtain the skin sample.

171. (New) The method of claim 167, wherein between one and twelve separate adhesive tape strips are applied to the skin to isolate the skin sample.

In re Application of:  
Rheins and Morhenn  
Application No.: 09/375,609  
Filed: August, 17, 1999  
Page 12

PATENT  
Attorney Docket No.: DERM1100-1

11 ~~172.~~ (New) The method of claim ~~157~~<sup>64</sup>, wherein the subject is a mammal.

12 ~~173.~~ (New) The method of claim ~~157~~<sup>64</sup>, wherein the subject is a human.

13 ~~174.~~ (New) The method of claim ~~157~~<sup>64</sup>, wherein the subject is a non-human.

175. (New) A method for quantitating relative expression of a ribonucleic acid (RNA) from an epithelium sample, comprising:

- (a) applying an adhesive tape to the epithelium, under conditions allowing for isolation of an epithelium sample adhering to the adhesive tape;
- (b) isolating RNA from other nucleic acids in the sample; and
- (c) comparing the level of the RNA in the sample to a control sample, thereby quantitating relative expression of the RNA in the sample.